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Tripartite mutualisms as models for understanding plant–microbial interactions

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All plants host diverse microbial assemblages that shape plant health, productivity, and function. While some microbial effects are attributable to particular symbionts, interactions among plant-associated microbes can nonadditively affect plant fitness and traits in ways that cannot be predicted from pairwise interactions. Recent research into tripartite plant–microbe mutualisms has provided crucial insight into this nonadditivity and the mechanisms underlying plant interactions with multiple microbes. Here, we discuss how interactions among microbial mutualists affect plant performance, highlight consequences of biotic and abiotic context-dependency for nonadditive outcomes, and summarize burgeoning efforts to determine the molecular bases of how plants regulate establishment, resource exchange, and maintenance of tripartite interactions. We conclude with four goals for future tripartite studies that will advance our overall understanding of complex plant–microbial interactions.

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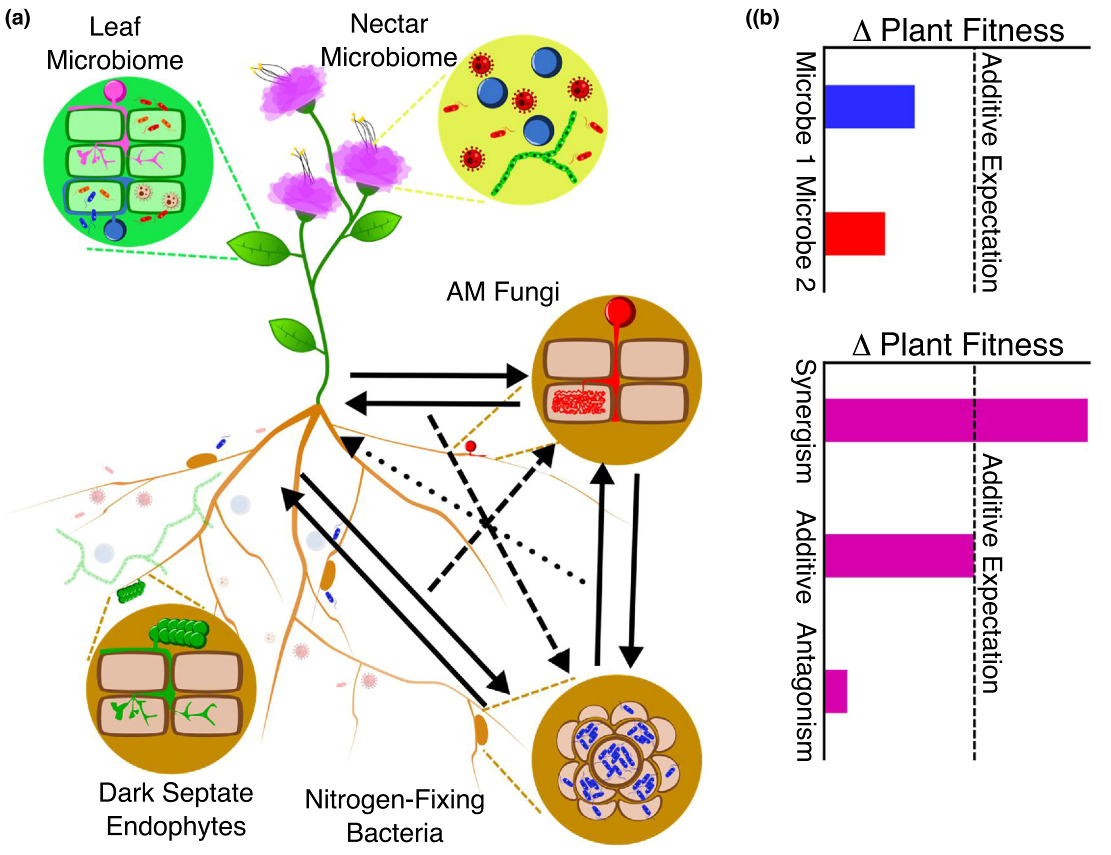
Microbes living in leaves, roots, flowers, and seeds play crucial roles in plant health and productivity (Figure 1a) [1,2,3•]. Decades of research has documented benefits from pairwise interactions between plants and 'microbes-of-large-effect' that provide limiting resources to plants [4,5] or protect against stressors [6,7]. A recent explosion of studies characterizing plant-associated microbiomes and their effects on host performance has further emphasized the role that microbes play in plant health [8–10]. While both pairwise and microbiome approaches have provided important insights into microbial effects on plant fitness, they represent two ends of a continuum. The pairwise approach allows the use of manipulative experiments to elucidate costs and benefits of interacting with microbes but cannot account for emergent, nonadditive effects that can occur as a result of interactions within the microbial community. Alternatively, microbiome studies embrace community complexity [9], but are often limited to correlative approaches or whole community inoculation studies to accommodate this complexity [11]. Applied efforts to utilize plant–microbial interactions in addressing the many challenges of the Anthropocene (e.g. improvement to sustainable agriculture) are hindered by a limited understanding of the ecological and molecular mechanisms underpinning plant responses to beneficial microbial consortia, though more holistic efforts are beginning to emerge [10]. Recently, research into tripartite plant–microbial mutualisms (associations between a plant and two microbial mutualist taxa) has made significant headway in addressing this challenge by marrying the strengths of a pairwise approach (i.e. detailed manipulations) and a microbiome-wide approach (i.e. inter-microbial interactions). In this review, we examine the current state of tripartite plant-microbe mutualism research. We discuss (1) nonadditive fitness effects in tripartite microbial mutualisms, (2) the context-dependency in these multispecies interactions, and (3) how molecular studies can elucidate the mechanisms that undergird fitness effects of tripartite interactions. Finally, we conclude by (4) highlighting four areas where future investigations of tripartite associations will advance our general understanding of complex microbial interactions with plants.

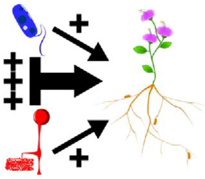
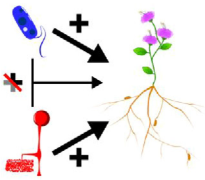
One plus one does not always equal two: fitness effects in tripartite mutualisms

Plants interact with many symbiotic microbes that can significantly affect plant fitness (Figure 1a). Some of these microbes may act independently, such that their effects on host plants are *additive* (Figure 1b; that is, individual microbial effects on plant fitness can be summed to determine their joint effect). However, many microbes have interactive, nonadditive effects on host fitness that range from *synergistic* (Figure 1b; that is, fitness with multiple microbes is greater than the additive expectation) to *antagonistic* (Figure 1b; that is, fitness with multiple microbes is less than the additive expectation)

Figure 1



(c)

Outcome for Host	Ecological Interaction Mechanism	Likely Effect on Microbial Partners	Direct vs Indirect effects on the microbes
Synergism 	Rewards conferred by microbes are complementary	(+/+)	Indirect
	Microbe-of-large effect (i.e. microbe providing important rewards to plants) depends on other microbe	+/?	Direct
	Competition among microbes for access to host conferred rewards leads to microbes providing more rewards to hosts than if host had no choice	-/-	Indirect
Additive	Microbes provide independent rewards and do not interact	0/0	None
Antagonism 	Competition among microbes generates pressure to exploit host (e.g. tragedy of the commons)	-/-	Indirect
	Competition among microbes diverts resources away from provisioning host to microbe-microbe warfare	-/-	Direct/Indirect

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Overview of fitness effects in tripartite plant–microbial mutualisms.

(a) Plants host a diverse assemblage of microbes in their leaves, shoots, flowers, roots, and seeds. Here we highlight one of the best-studied tripartite microbial mutualism, which is between host plants, nitrogen-fixing bacteria, and arbuscular mycorrhizal (AM) fungi. Beneficial microbes can interact directly with their host plant as well as with other microbes in the community (indicated by solid arrows). The microbes can also

[12,13]. Plants can experience synergistic fitness effects in a tripartite mutualism if the persistence of microbes-of-large effect depends on other members of the microbial community, or if the microbes provide complementary rewards to the host plant (Figure 1c). For example, arbuscular mycorrhizal (AM) fungi and rhizobia had a synergistic effect on root biomass of the legume *Amorpha canescens* such that when grown with both microbes, root mass was 280% greater than the additive expectation [14]. Synergistic effects of AM fungi and rhizobia on plants are *not* universal [13], but have been documented repeatedly in recent years [15–17], likely because these two microbes-of-large effect can provide complementary resources important for plant growth (e.g. phosphorus/ water and nitrogen, respectively). Beneficial effects of multiple microbes can also arise through synergistic suppression of pathogens and herbivory. Recently researchers documented mycorrhizal fungi and a plant growth-promoting rhizobacteria (*Pseudomonas putida*) synergistically increased immune system priming in wheat [18]. Similarly, another study found that when grown with two beneficial rhizobacteria (nodule-forming *Bradyrhizobium japonicum* and plant growth-promoting *Delftia acidovorans*) soybean had reduced intensity of the pathogenic bean pod mottle virus and increased plant fitness compared to plants inoculated with either mutualist alone [19]. Interestingly, synergistic effects can occur even when a microbe is not a mutualist in isolation (i.e. when participating in a pairwise interaction). Dual inoculation of the herb *Verbascum lychnitis* with both fungal root endophytes and AM fungi, for instance, had synergistic effects on plant biomass and survival rates [20]. While endophyte effects on the plant were often negative when introduced alone, they enhanced plant performance in combination with AM fungi. These results show that tripartite interactions can not only alter the strength, but also the direction of microbial effects on host plants. Antagonistic effects on plant performance have been documented as well [21•,22]. These effects may be driven by carbon limitation of plants in low light environments [23] or by competition among microbes [24,25] that causes overexploitation of host resources (e.g. tragedy of the commons) or diversion of resources away from the host plant to instead be used in microbe-microbe warfare.

Underpinning these nonadditive fitness effects on hosts are direct and indirect inter-microbial interactions (Figure 1c). In addition to *direct* interactions between plant-associated microbes (e.g. competition for space or environmental resources [24,25]) that may be facilitated by the close proximity encouraged by life on/within plants, many microbes interact *indirectly* by modifying traits of their shared host (Figure 1a). Microbes that induce changes in root structure [26], allocation to roots versus shoots [27], and chemical composition of plant tissues and exudates [28] can substantially affect other microbial mutualists. Further, microbes may influence one another through resource trade with their hosts (see Box 1). For example, under low phosphorus availability, alfalfa and its wild relative *Medicago truncatula* inoculated with AM fungi exhibited significantly higher nitrogen-fixation rates by rhizobia compared to non-mycorrhizal plants [29]. The AM fungi increased phosphorus availability to the plant, which the authors hypothesize was shared with rhizobia, removing phosphorus-limitation inhibiting nitrogen-fixation. Positive indirect effects driven by resource trade occurs when microbes that provide complementary rewards to host plants cause increased plant performance, such that there is an increase in quality/quantity of resources available for provisioning one or more of the microbes (Figure 1c). Microbes can also inhibit one another through indirect interactions by inducing changes in the plant phenotype that are detrimental to the association with another partner or through competition for a limited supply of photosynthetic carbon (Figure 1c; [30]).

Recent tripartite studies have also explored the role of biotic and abiotic context-dependency in determining effects on plants, showing that nonadditivity only occurs under some conditions. While our understanding of how abiotic factors influence tripartite microbial-plant interactions is much less developed than for bipartite interactions (bipartite reviews: [31,32]), many important abiotic contexts have been examined, including temperature [33], salinity [34], nutrient concentration [35•], and availability of water [36] and light [23]. In addition to its importance for determining when microbial effects will be nonadditive, abiotic context can shift when these interactions are beneficial versus costly to the host. For example,

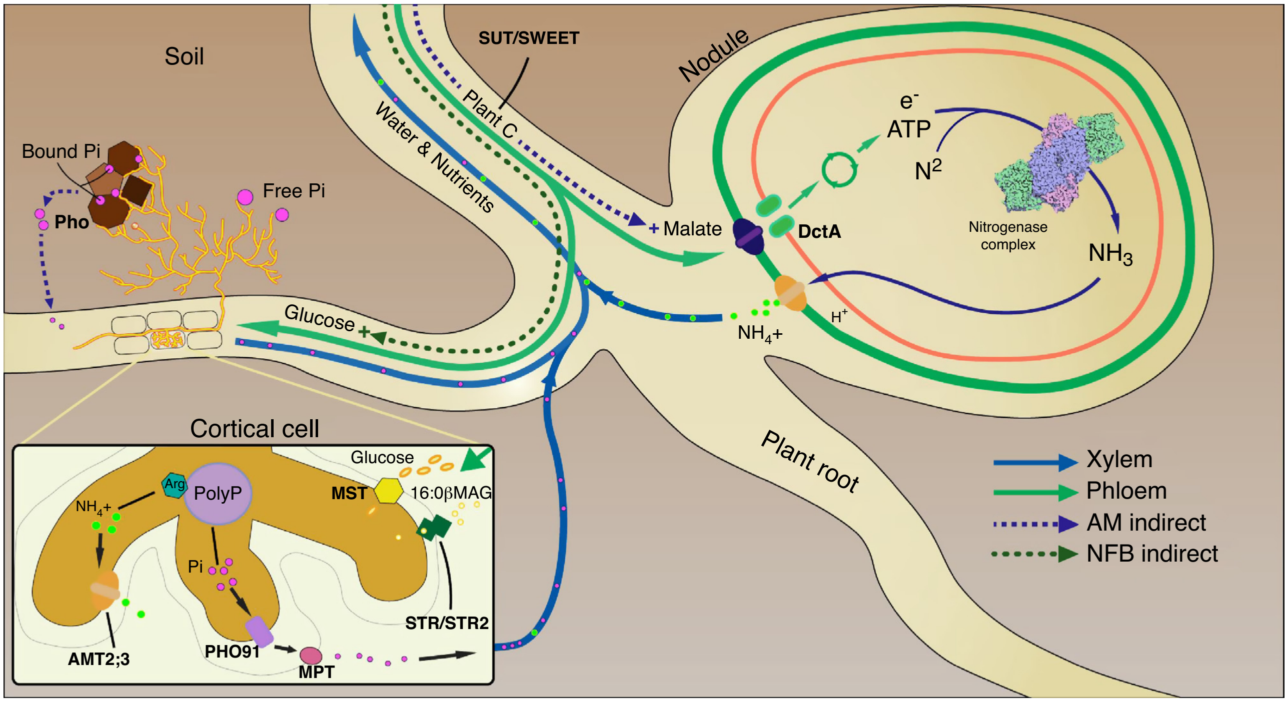
(Figure 1 Legend Continued) interact indirectly through changes in their shared host (indicated by dashed arrows) and a microbe can indirectly affect the host plant through changes to another microbe (indicated by dotted arrows). **(b)** The effect of multiple microbes on plant fitness may be additive (i.e. the sum of the individual microbial effects on plant fitness equals their joint effect) or nonadditive, ranging from synergism (i.e., fitness with multiple microbes is greater than the additive expectation) to antagonism (i.e., fitness with multiple microbes is less than the additive expectation). Red and blue bars represent plant fitness when grown with only ‘Microbe 1’ or only ‘Microbe 2’, respectively. Purple bars represent possible outcomes of tripartite interaction for plant fitness. In both graphs, the dashed line represents the additive expectation based on the fitness effects of Microbes 1 and 2 in isolation. **(c)** For each of these outcomes (synergism, additive, and antagonism), this table describes a set of ecological mechanisms/explanations that could generate that outcome for the host as well as the likely effect on the microbial partners. It also indicates whether the effects on the microbes are expected to result from direct or indirect pathways. Likely effects written as ‘(+,+)’ indicate that while complementarity between microbial partners on host fitness is expected to positively feedback on microbes, how plants will allocate additional resources is context-dependent.

Box 1 Biological market theory and mechanisms of tripartite mutualism functioning

‘Biological markets’, in which goods and services are exchanged between cooperating species, have served a central role in understanding mutualistic interactions [51]. Using the Biological Market Theory (BMT) framework, researchers can harness tangible measures of multiple goods (e.g. plant carbon, mycorrhizal phosphorus, rhizobial nitrogen) and services (e.g. pathogen resistance, resource restriction, or competitive exclusion) in mutualistic interactions and determine how specific partner interactions alter fitness outcomes. BMT has been used to identify the importance of mutualistic partner selection and reciprocal trade between partners as well as empirically detail the exchange patterns of key resources amongst plant and microbial partners [52,53]. While BMT has, to date, predominantly provided insight into the specific controls within bipartite mutualisms, it also holds promise for understanding incentives in tripartite interactions.

Application of BMT to tripartite mutualisms requires mechanistic understanding of relative values and costs of resources mediated by all of the partners involved. For example, in the AM fungi-rhizobia-legume market in which sugar is the basic symbiotic currency, recent characterization of microbial nutrient transporters has provided insight into direct and indirect effects of partners on one another (Figure 1a, Box Figure I). Decades of research has revealed the genes and transcription factors that detail pathways of carbon flow from plant hosts and nutrient exchange sites of AM fungi and rhizobia (Box Figure I). Interpretation of nutrient transfer in plant–microbial mutualisms is often based on metrics of plant or microbial biomass (particularly for obligate AM fungi) or analysis of plant nutrient concentrations, but these measures cannot easily capture the ‘when and where’ of plant C allocation to microbes, or the potentially reciprocal nutrient transfer from mutualists to the plant. Measuring gene expression of transporters involved in resource exchange could allow researchers to more precisely track the trade market among the three partners, particularly when growth-limiting nutrients alter resource trade in the AM fungi-rhizobia-legume mutualism. A recent study demonstrated that when plant N-demand was higher, more C was allocated to rhizobia than AM fungi, and conversely more C was allocated to AM fungi than rhizobia when N-demand was lower, and that these allocation patterns were correlated with expression levels of mutualist-specific plant sucrose transporters, namely MtSUT2, MtSUT4, and MtSWEET11 [54•]. Inter-microbial interactions might also be better explored using a trade-tracking approach in the AM fungi-rhizobia-legume model system where N_2 fixation is a notably P-demanding process. Nodules are P-sinks and P deficiency can inhibit N_2 fixation [55]; therefore, AM fungi may indirectly mitigate P limitations to rhizobial N fixation. This can happen via increased P allocation to the plant host [29], and indirectly via mycorrhizal phosphatase-induced release of bound inorganic phosphorus (Pi) from the soil matrix (Box Figure I, blue dashed arrows). Utilizing fine-scale measures of microbial resource trade markets in factorial multiple-mutualist studies will help us better understand direct and indirect mechanisms shaping tripartite mutualisms.

Figure I



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Microbial trade pathways and mechanisms in a tripartite mutualism.
After nodule formation by rhizobia (right) and root colonization by AM fungi (left), expression of sugar transporters (SUTs, SWEETs) in host plants increases. For AM fungi, monosaccharide transporter genes (MST) upregulate with glucose, and STR/STR2 transporters upregulate with fatty acid (16:0βMAG) transfer from the plant at the arbuscule-plant interface (inset box). Plants take up inorganic P (Pi) directly via mycorrhiza-induced Pi transporters (MPT), and indirectly acquire previously bound Pi from soils after hyphal proliferation and release of acid phosphatases (Pho). For nitrogen-fixing bacteria, the dicarboxylate transporter (DctA) takes in malate from plants, and then utilizes ATP and N_2 to create NH_3 via an nifHDK complex. Bacterioids then secrete NH_4+ to the plant. Indirect trade pathways between microbial partners are mediated through potential increases in plant C after Pi allocation from AM fungi (AM, blue dashed arrows) or NH_4+ allocation from nitrogen-fixing bacteria (NFB, green dashed arrows).

the effects of multiple endophytic fungi on switchgrass (*Panicum virgatum*) shifted from antagonistic to synergistic depending on soil moisture, such that plants were more likely to experience delayed leaf wilt in wetter conditions when grown with some, but not all, pairs of endophytes [21•]. The abiotic context also modulates effects of microbes on one another. For instance, the plant-growth promoting rhizobacteria *Pseudomonas putida* increased nodule formation of the nitrogen-fixing bacteria *Bradyrhizobium japonicum* by ~50% under no salt or low salinity compared to 75% under high salinity [34], and in another study, the presence of AM fungi was required for nodulation under the highest level of sulfate salinity [16].

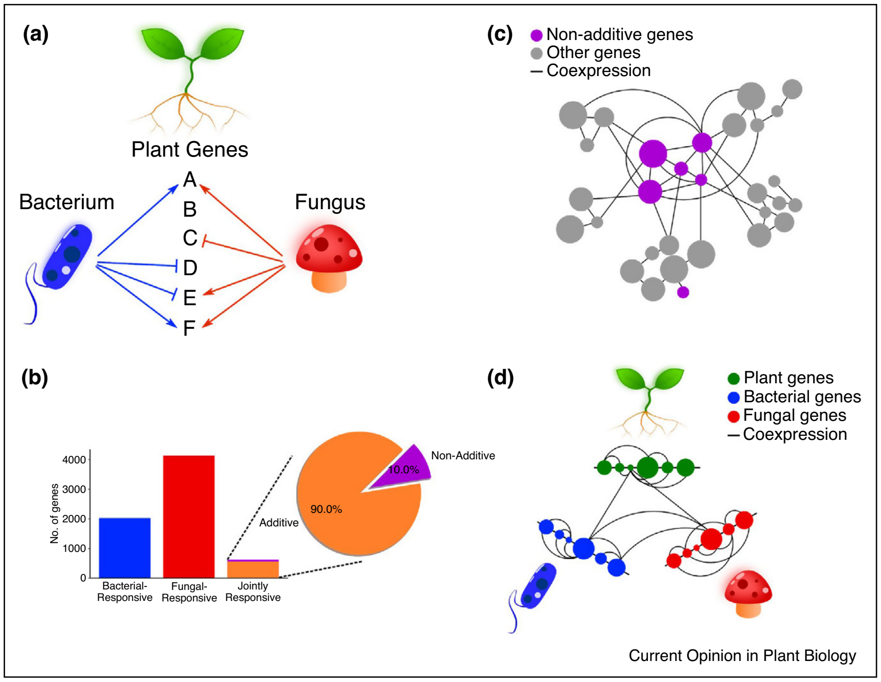
Many biotic contexts that are 'external' to the organisms participating in the tripartite interaction, such as strength of pathogen pressure or composition of the herbivore community, can significantly alter the outcome of these interactions [37,38]. In addition, the host's species and/or genotype identity has been identified as an 'internal' biotic context influencing the outcomes of tripartite associations (e.g. cultivar-specific synergistic effects of AM fungi and *Bradyrhizobium japonicum* on height, biomass, and seed yield of soybean [39]). These context-dependent effects may be caused by variation in the abilities of plant genotypes/species to differentiate among microbes of varying quality and/or exert host controls to regulate them. Species and genotype identity of the microbes can also play a key role in determining when nonadditivity happens, with the specific combination of strains used in co-inoculation experiments impacting the outcome for plant fitness. Recent work documented that deviations from additive expectations for effects of dual endophyte inoculations on switchgrass growth was largely predictable based on fungal niche overlap, defined as dissimilarity among fungal traits in culture [21•]. In line with the theoretical expectation that functionally distinct microbial partners can provide complementary rewards (thereby underpinning host synergism), co-inoculation with less similar endophytes typically had more synergistic effects on plant growth. Interestingly, they also found that the majority of the nonadditive responses to co-inoculation were synergistic effects on switchgrass performance (79% synergistic rather than antagonistic effects). Overall, the genotype/strain-specific responses in tripartite interactions strongly suggest that genomic variation in the host and microbial partners contributes to the realized fitness effects organisms experience in tripartite mutualisms.

Good relationships are complicated: genomic perspectives into mechanisms underpinning tripartite mutualisms

While most genomic research on beneficial plant-microbe interactions focuses on pairwise associations, the mechanistic basis of microbial effects on plants and (to some extent) the participating microbes has benefited from recent work using genomic approaches to study tripartite interactions. Most studies of genomic and molecular mechanisms in tripartite plant-microbe associations have used the well-established AM fungi-rhizobia-legume symbiosis. Advancement in this field is best embodied in the 'Common Symbiosis Pathway', a plant signalling cascade important for *establishing* symbiosis with both rhizobia and AM fungi, reviewed extensively in [40,41•]. In short, both rhizobia and AM fungi release diffusible chemical signals in the rhizosphere recognized by root cells that then initiate calcium-spiking and a release of secondary messengers, ultimately resulting in nodule formation or arbuscule development. This foundational research spotlighted the genetic and mechanistic overlap in symbiotic establishment pathways [42,43•]. More recently, studies have begun to examine mechanisms that *maintain* tripartite mutualisms by identifying and measuring the genetic controls of nutrient transfer between plants and rhizobia [5] or AM fungi [4]. In Box 1, we highlight the potential of complementing these functional and mechanistic assays with "Biological Market Theory". Integration of molecular biology and ecological theory can both identify biologically meaningful reasons plants participate in multispecies microbial mutualisms as well as how they establish, maintain, and sanction them.

While knowledge of these symbiotic pathways has provided important mechanistic insight into establishment and trade within tripartite interactions, these pathways and known upstream and downstream genes constitute a relatively small fraction of the plant genome. Much cross-talk between different biochemical pathways that regulate tripartite mutualisms likely remains to be discovered and characterized. Increasing accessibility of genome-wide expression tools (e.g. RNA-Seq, ChIP-Seq, etc.) has provided an opportunity to examine the complex responses to tripartite interactions. For example, in a recent factorial differential expression study, *Medicago truncatula* plants were grown in the presence of both rhizobia and mycorrhizal fungi, with each microbe individually, and with no microbes (Figure 2; [17]). Results revealed >600 plant genes whose expression were jointly affected by the presence of both mutualists of which 62 genes' expression levels were affected nonadditively by the microbes (Figure 2b; [17]). In addition to being enriched for nutrient acquisition functions such as acid phosphatase activity and glutamine synthesis, both the additively and nonadditively affected genes were significantly more central (i.e. connected) within coexpression networks than expected by chance (see conceptual diagram in Figure 2c; [44•]). In fact, the 62 nonadditive genes were 94% more central than even the additive genes. In network analyses from other systems, high centrality has been used to detect biological importance for coordinating responses within the network [45–47], suggesting that the nonadditive genes are playing important roles in coordinating plant molecular responses to multispecies

Figure 2



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Effects of tripartite plant–microbial mutualism on genome-wide expression.
(a) Mutualists can induce or repress gene expression in their host plants and, thus, genes can be responsive to only one mutualist (e.g. genes C and D are fungal- and bacterial-responsive, respectfully) or affected by both mutualists (e.g. genes A, E, and F). In cases where host genes are targeted by both microbes, mutualist effects can agree in direction such as genes A and F where both microbes induce their expression or mutualist effects can disagree such as for gene E for which one microbe (bacteria) represses expression and another (fungi) induces it. **(b)** In a recent differential expression study of multiple mutualist effects in *M. trunctatula* (the legume barrel medic [17]), plants were inoculated with both the rhizobacterium *E. meliloti* and the AM fungus *R. irregularis*, each microbe separately, or no microbes. The change in plant gene expression in the tripartite context was compared to the sum of the change in gene expression in both pairwise inoculations to determine single-mutualist-responsive genes and jointly responsive genes. Most differentially expressed genes were responsive to only one mutualist (bacteria or fungi; blue and red, respectively). A small subset (623 genes) were affected by both bacteria and fungi and within this subset 90% were affected additively (orange) and 10% nonadditively (purple). **(c)** Conceptual diagram of the position of nonadditive genes in a tripartite inoculation coexpression network of plant gene expression based on [44•]. Nonadditive genes were highly connected in their networks (i.e. they were coexpressed with many other plant genes) with ∼3× more connections than randomly sampled genes. **(d)** Conceptual diagram of ‘across-symbiosis’ coexpression. Coexpression Network of each participant is colored (plant = green; bacterium = blue; fungus = red) and linearized. Expression of genes from different mutualists may also occur in tandem and thus form across-symbiosis coexpression modules. Biologically, across-symbiosis coexpression suggests that the genes of one partner may regulate the expression of another partner.

microbial interactions. Therefore, these genes — especially the smaller pool of nonadditive genes — are interesting candidates for how plant condition is affected by tripartite interactions and how plants regulate these multispecies associations. These studies can also provide insight into responses of partner microbes. In a recent paper, the presence of a third-party mutualist, rhizobia *Ensifer meliloti*, caused significant rewiring of coexpression within AM fungi gene modules that were enriched for important mycorrhizal metabolic and cellular processes such as metabolism of *N*-acetylglucosamine, a molecule important to early signalling between plants and AM fungi [44•,48]. The rhizobia also caused significant changes in coexpression relationships across the symbiotic boundary within modules containing both host plant and AM fungi genes that showed-coupled host-symbiont functions (Figure 2d; [44•]). For example, a plant-fungal module associated with lipid transport (i.e. carbon transport) in plant genes and potassium ion transport (i.e. nutrient transport) in AM fungi was rewired in the presence of *E. meliloti*. These results indicate that utilizing networks that examine coexpression of genes within partner microbes and across the symbiotic boundary (i.e. coexpression between genes in host plants and a partner microbe) provides a profitable avenue for understanding the inter-microbial dynamics of host-symbiont interactions in the future.

Conclusions and future directions

As we have illustrated in this review, recent work on tripartite microbial mutualism has meaningfully improved our understanding of how microbes affect plant performance and identified mechanisms that underpin these interactions. However, large knowledge gaps still exist. Below we conclude this review by highlighting four goals for future investigation that will be crucial building blocks in developing a comprehensive understanding of plant–microbial interactions:

- **Determine direct and indirect pathways underlying inter-microbial dynamics.** While studies have shown that microbes in tripartite interactions can affect one another, the pathway through which this happens remains unknown in the vast majority of cases. Elucidating *direct* and *indirect* interaction pathways and their relative importance for plant and microbe performance is critical for developing a predictive framework for nonadditive fitness responses. In addition to studies that track rewards and changes in molecular phenotypes (see goals below), research explicitly linking shifts in host functional traits driven by one microbe to changes in colonization, performance, and function of another microbe are needed to identify and characterize indirect pathways.
- **Track trade in tripartite mutualisms.** The relative value of rewards transferred among plants and microbes in a mutualistic trade market determine the productivity and, ultimately, the fitness of each partner involved. Utilizing measures of nutrient transfer mechanisms (e.g. sugar, Pi, and NH_4 transporters; Box 1) to track shifts in allocation patterns could substantially advance our understanding of both synergism and nonadditivity in AM fungi-rhizobia-legume interactions, especially if the metrics are assessed in experiments that factorially manipulate the presence of each microbe along N and P availability gradients. Ideally, similar efforts to track rewards should be completed for tripartite interactions involving other types of benefits (e.g. salinity/thermal/drought tolerance, herbivore/pathogen defense).
- **Identify and tie genes to functions involved in regulation and maintenance of tripartite interactions.** Biochemical pathways involved in *establishment* of the model tripartite interaction between legumes, rhizobia, and AM fungi have been characterized using mutagenic assays. However, the genomic basis of processes underlying how tripartite interactions are *maintained* and *regulated* across host ontogeny remains less clear. Broad surveys via GWAS and RNA-Seq are powerful tools to generate-specific hypotheses about these genes, but are scarce for multispecies interactions. We encourage broader use of these approaches across multiple contexts and stages of these complex interactions. Mutagenic assays (e.g. gene knock-out/in studies) should be used in tandem for validation, directly tying genes to observable impacts on the tripartite association. Further, expanding these studies outside of AM fungi-rhizobia-legume associations is crucial for identifying the conserved versus unique molecular mechanisms that plants and microbes employ to regulate these multispecies interactions [43•,49].
- **Scale up effects of tripartite interactions to population, community, and ecosystem-level processes.** Given the substantial nonadditive effects that microbe-microbe interactions have on plant performance/traits at the individual level, we encourage investigation into how these effects can scale up to impact population, community, and ecosystem level processes. These higher order consequences have rarely been investigated [3•,50], leaving expansive ground for future work. For example, what are the consequences of tripartite interactions for population dynamics of microbes, plants and other organisms (e.g. herbivores and pollinators), their community assembly, and the stability and abundance of ecosystems services (e.g. nutrient cycles)? Understanding these and other processes in a tripartite context could enhance our understanding of plant–microbial mutualisms and inform applied efforts to utilize plant-microbe interactions in sustainable agriculture and bioremediation.

To move beyond the limitations of descriptive and pairwise studies and achieve the ambitious research goals we have outlined for tripartite interactions, integrative approaches that scale from molecular and functional mechanisms to higher order ecological processes will be required. Thus, collaborations within and across research groups leveraging expertise from a diversity of fields and levels of biological organizations will be a crucial part of developing holistic perspectives on plant–microbial interactions in the future.

Conflict of interest statement

Nothing declared.

Author contributions

All authors contributed equally to this manuscript. MEA conceptualized and led the construction of this review; DJH, DPR, and MEA made the figures, and all authors wrote the manuscript.

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This study examined the effects of multiple microbial mutualists on microcosms of grassland plant communities. Using 11 plant species from the three main functional groups (herbs, legumes, grasses) in the grassland system, they demonstrated co-inoculation of AM fungi and rhizobia had complementary effects, resulting in increased plant diversity, seedling recruitment, and nutrient uptake compared to microcosms with a single mutualist (i.e. either AM fungi or rhizobia). This study shows the importance of investigating multiple microbial partners, especially those that provide functionally different services, in a community context and outlines an exceptional experimental design to begin asking further questions about the impacts of multiple mutualisms on community dynamics/assembly and ecosystem functions.

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This paper takes an evolutionary perspective on tripartite mutualisms, using a quantitative genetic approach to investigate whether the pleiotropy identified in the plant genetic pathways for colonization of rhizobia and AM fungi shapes evolution in natural populations. They grew plants from 75 maternal families of the legume *Chamaecrista fasciculata* under two phosphorus environments and examined plant genetic covariance between rhizobia and AM fungi colonization. They found little evidence for genetic covariance in either high or low phosphorus environments, which they hypothesize means that genetic variation at other, nonpleiotropic loci governs variation in colonization and that colonization by rhizobia and AM fungi evolves independently in these plant populations. This work highlights the need to incorporate an evolutionary perspective and toolkits into tripartite studies moving forward.

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This review focused on the extensive signaling, cellular remodeling, and metabolic changes that occur in the plant-AM fungal symbiosis, and developed a comprehensive picture of the Common Symbiosis Pathway shared by both rhizobia and AM fungi. They present the current state of knowledge of how plants fine-tune their physiological and cellular state to enable and maintain microbial endosymbionts. This review sets the stage for future work investigating the potential commonalities among plant hosts with different evolutionary trajectories, and suggests that more studies specifically targeting the microbial side of the symbiosis need to be carried out.

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This paper examined how *Medicago truncatula* carbon allocation to root symbionts shifted in tripartite interaction under different nutrient supply conditions. They tracked productivity and nutrient concentration as well as expression of the SUT and SWEET carbohydrate transporters genes. Under nitrogen-limitation, plants allocated more carbon to rhizobia than AM fungi, but this pattern was reversed when nitrogen-limitation was alleviated. Importantly, SUT and SWEET gene expression was correlated with plant allocation to the respective microbial symbiont. This study illustrates how plants can exert fine-scale regulation of their interactions with microbial mutualist partners in direct response to resource availability, where microbial rewards that best remove limitations to productivity are preferentially allocated vital photosynthate. Few, if any, studies have performed this analysis with a plant in *tripartite* mutualism, and the findings bolster our understanding of mutualist reward complementarity.

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